Cancer of the rectum colon: high FeCl3 content inhibits the genotoxic activity colibactin produced by E.Coli strains.

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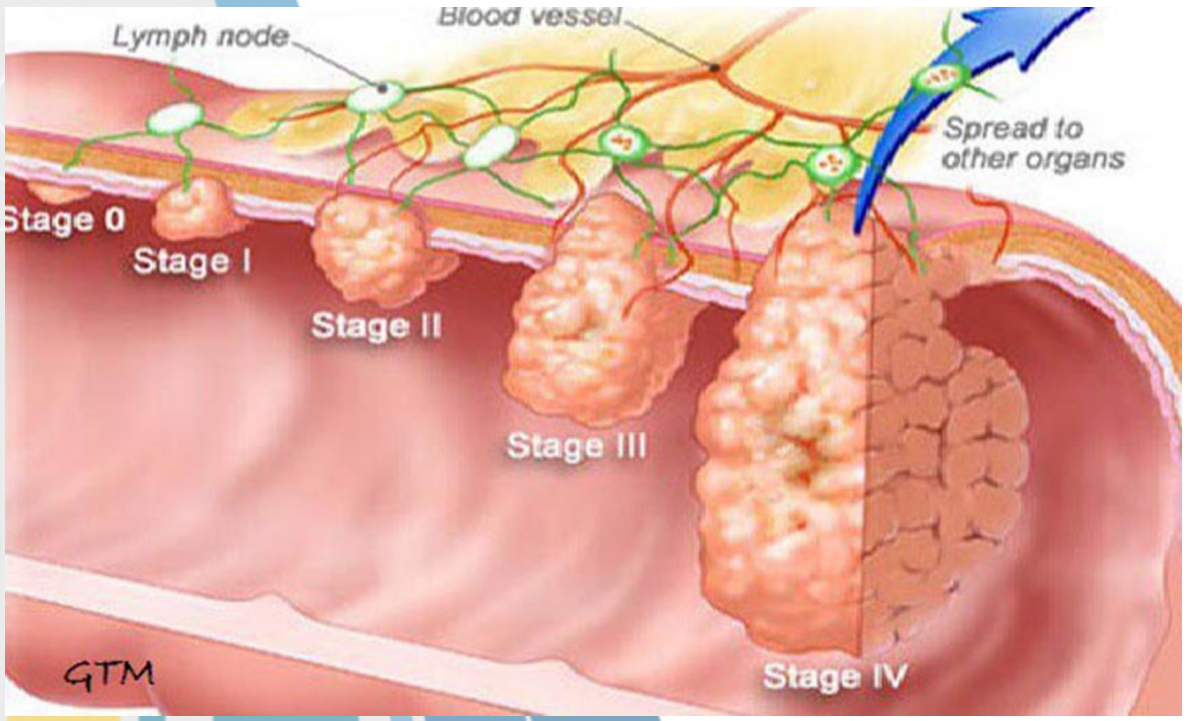
RECTAL COLON CANCER

Colorectal cancer (CCR) is one of the most frequent neoplasms in Italy. It is very present in developed countries, where more than 65% of cases are found.

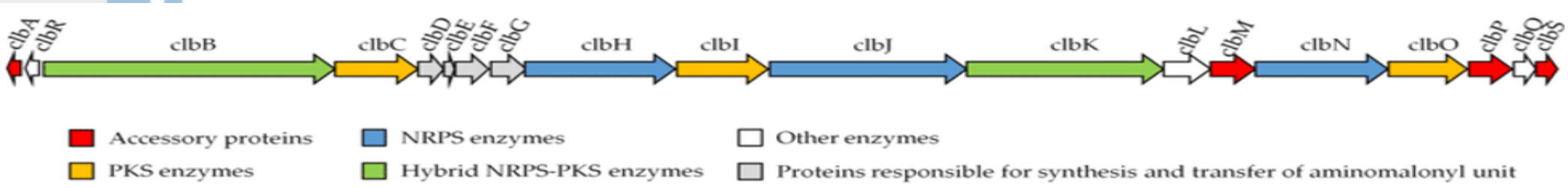
Rank	Men	Women
1°	Lung (27%)	Udder (17%)
2°	**Rectum Colon(10%)**	**Rectum Colon (12%)**
3°	Prostate (8%)	Lung (11%)
4°	Liver (7%)	Pancreas (7%)



It can be caused by both intrinsic and extrinsic factors, such as:

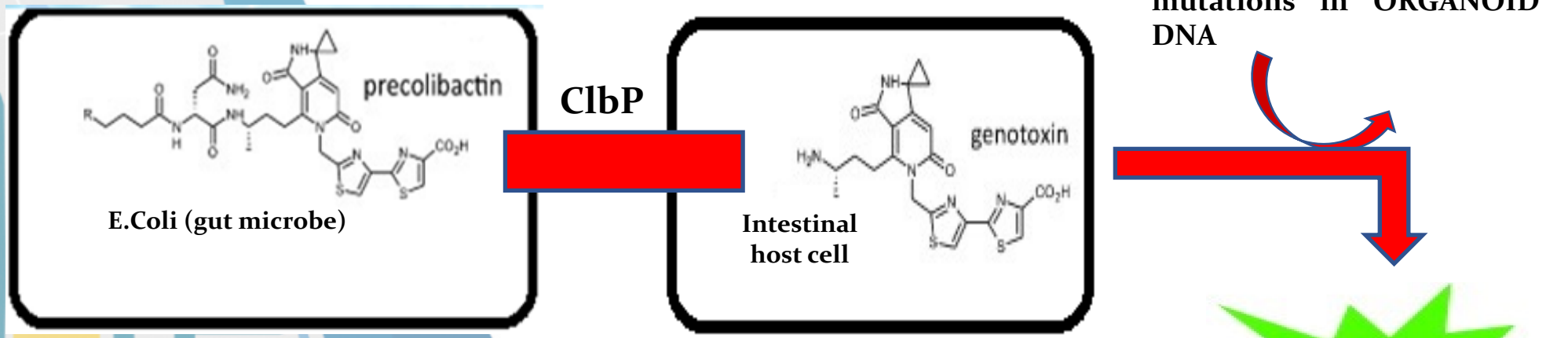
- Bad power supply;
- Smoking and alcohol;
- Age ;
- Genes Factors;
- **Intestinal strains of E.Coli.**

PKS island features



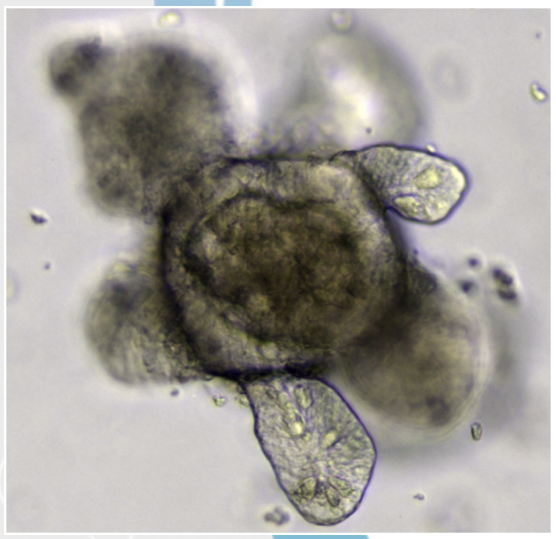
- PKS island codify for many proteins having a different role in the production and activation of the **COLIBACTIN**;
- The **COLIBACTIN** transforms healthy stem cells into cancer stem cells.

HOW THE COLIBACTIN ACT INSIDE HOST CELLS?



Interaction between Bacteria and Intestinal host cell

ORGANOID'S FEATURES



- 3D *in vitro* culture systems;
- Can be developed from pluripotent stem cells and adult stem cells;
- Used to study multiple organs as intestine, brain etc;
- Used in multiple clinical applications including **host–microbe interactions;**
- The complex interplay between microbes (bacteria, parasites, viruses)

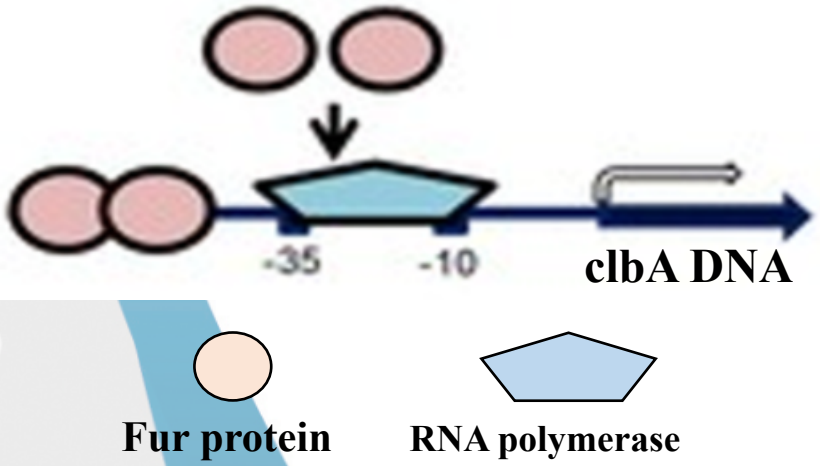
and the host epithelium have been dissected using organoids derived from intestine.

ABOUT 3D PRODUCTION.....

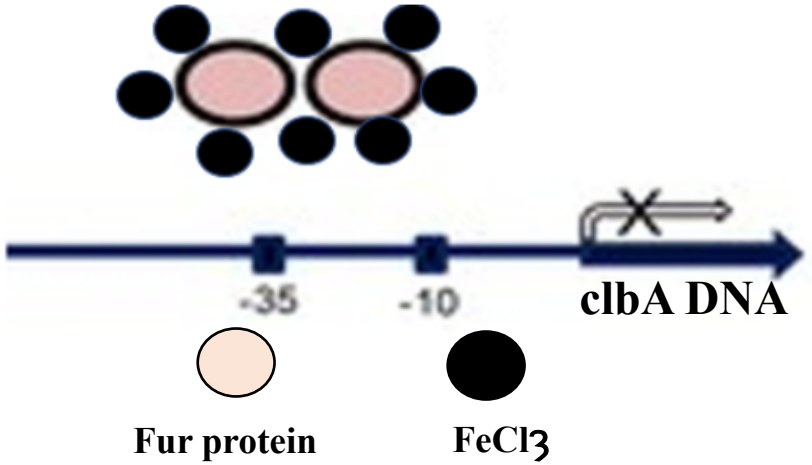
- Intestinal tissue biopsy;
- Pick stem cells from tissue;
- Put the stem cells inside grow medium;
- *In vitro* proliferation

AIM OF PROJECT

Absence of FeCl3



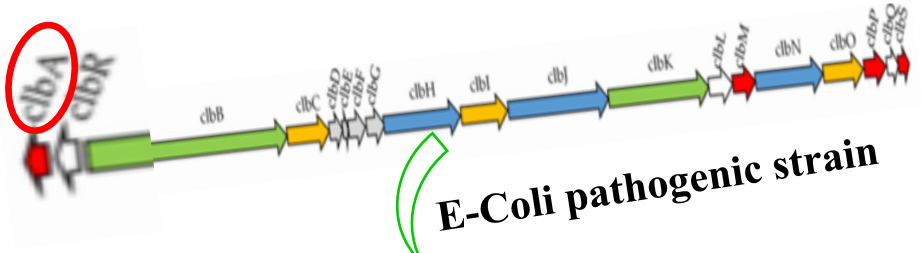
Presence of FeCl3



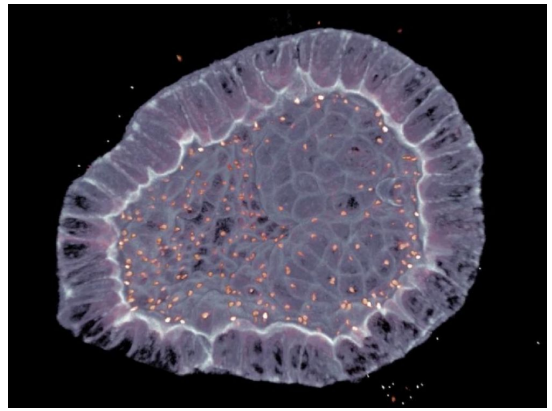
Regulation of Colibactin by using Iron:

- In absence of **FeCl3** Fur bind the promoter of clbA gene and the transcription is activated.
- In presence of **FeCl3** Fur can't bind the promoter of clbA gene and the transcription is repressed;

HOW?



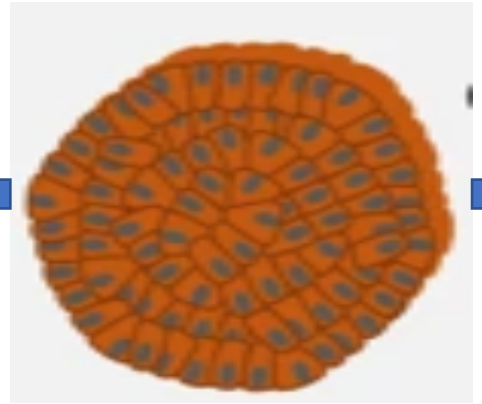
E-Coli pathogenic strain



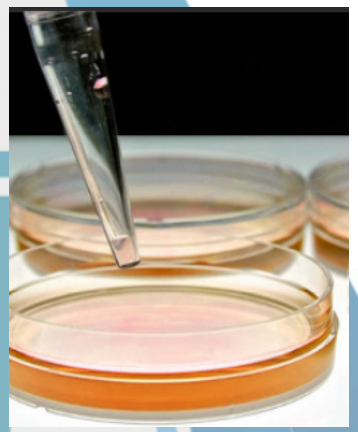
ORGANOID

+ FeCl3

- FeCl3



Co-Colture + DMEM Hepes

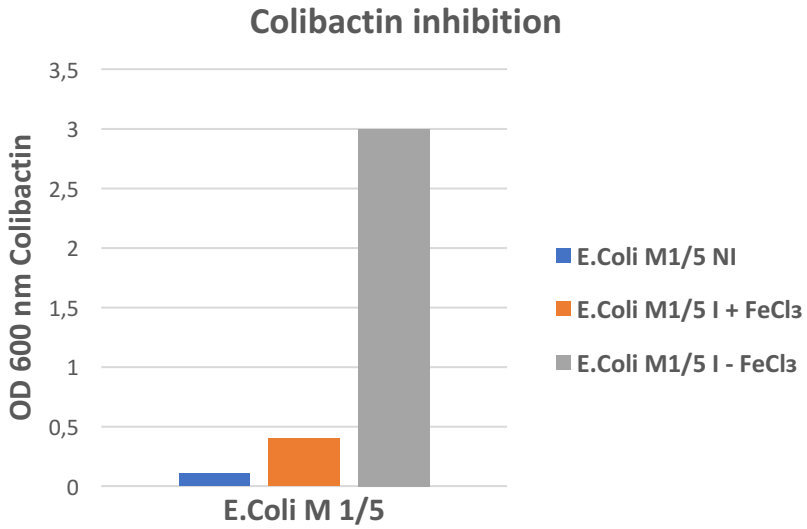


Co-Colture + FeCl3

Co-Colture - FeCl3

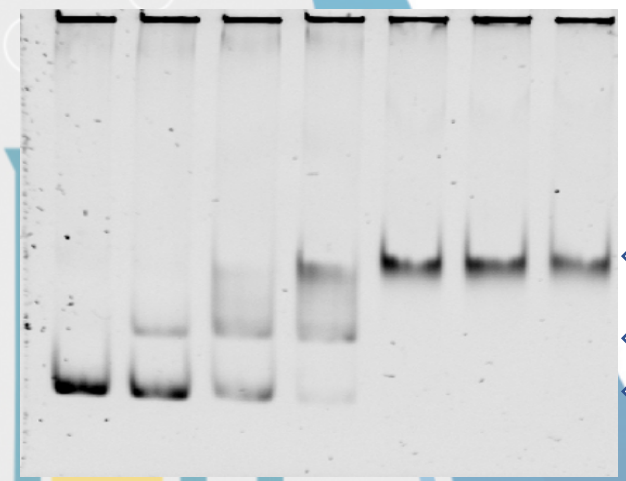
IN VITRO EXPERIMENT

- ✓ ORGANOID + STRAIN M1\5 WT + Fur
- ✓ DMEM CULTURE MEDIUM



- ✓ ORGANOID + STRAIN M1\5 WT + Fur
- ✓ DMEM CULTURE MEDIUM
- ✓ 100ϱM DI FeCl3

Fur - FeCl3



B_2

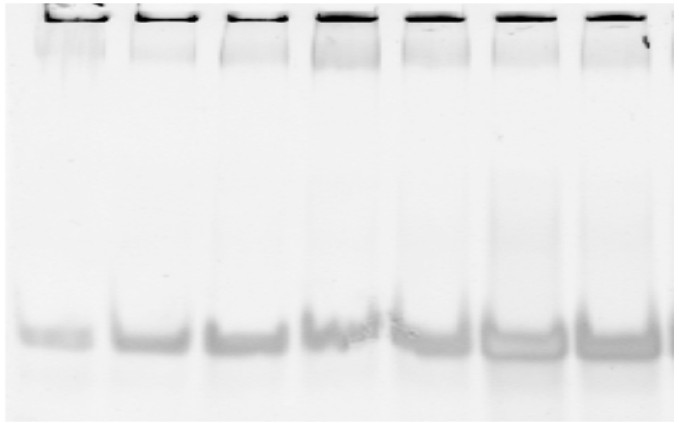
B_1

clbA promoter

1) Probe promoter of clbA 2-7) Probe promoter of clbA incubated with increasing amount of Fur protein in absence of FeCl3

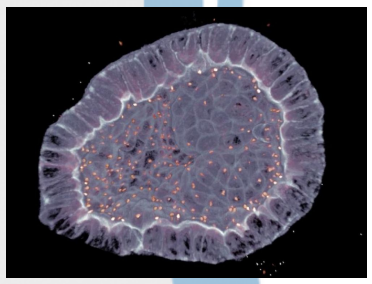
Fur + FeCl3

clbA promoter



1) Probe promoter of clbA 2-7) Probe promoter of clbA incubated with increasing amount of Fur protein in presence of FeCl3

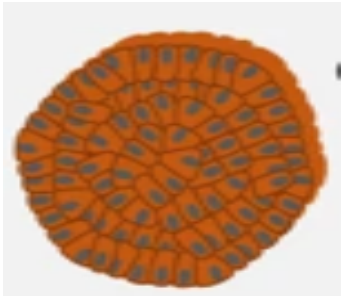
Regulation of Colibactin production by FUR protein in absence of **the FeCl3**



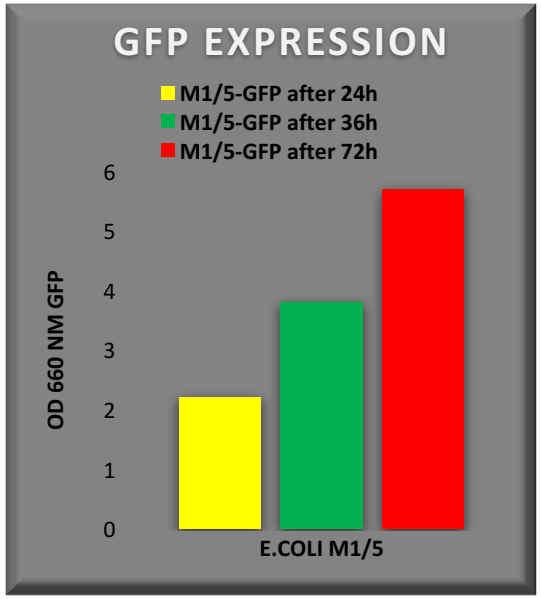
ORGANOID

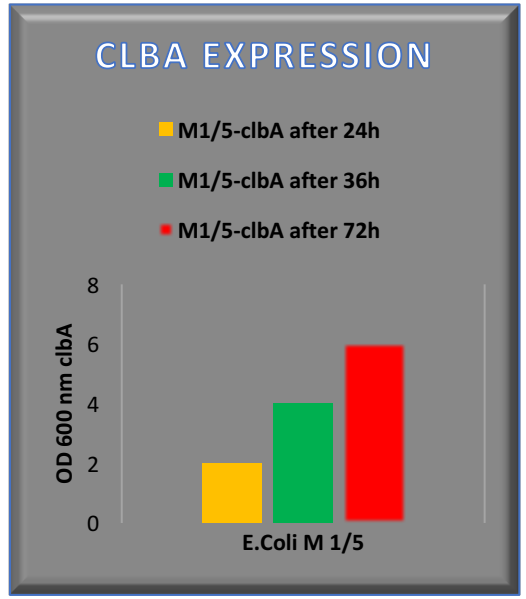
-35 -10 | clbA | GFP

Dna sequence of E.Coli

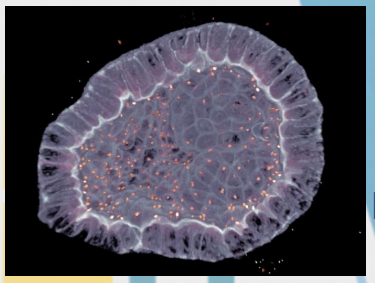


ORGANOID INFECTED





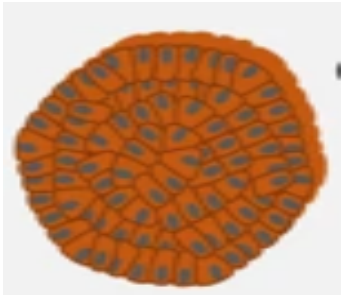
Regulation of Colibactin production by FUR protein in presence of **the FeCl3**



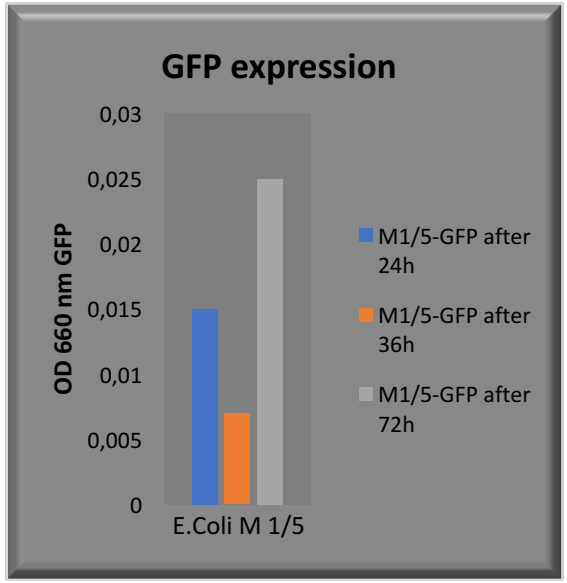
ORGANOID

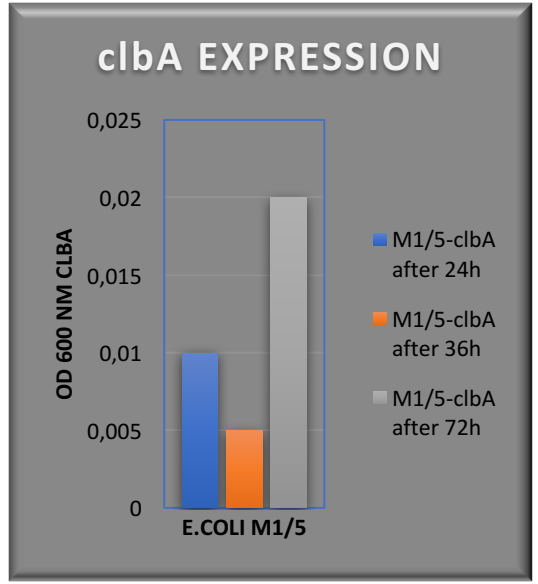
-35 -10 | clbA | GFP

Dna sequence of E.Coli



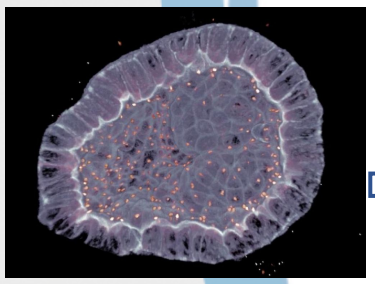
ORGANOID INFECTED





HOW?

IN VIVO EXPERIMENT



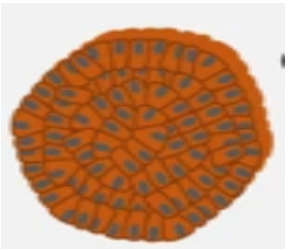
ORGANOID

-35 -10	clbA	GFP	Amp

Dna sequence of E.Coli

Co-Colture + DMEM
Hepes +AMP

Cells isolation



ORGANOID
INFECTED

Microinjection of the infected organoid into isolated embryo

Host Mice

Coupling and waiting up to 3 days

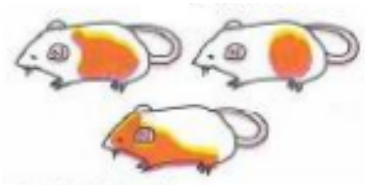
Isolated embryo in early stage of development

Early embryo composed to infected organoid

Introduction of the early embryo in pseudopregnant female

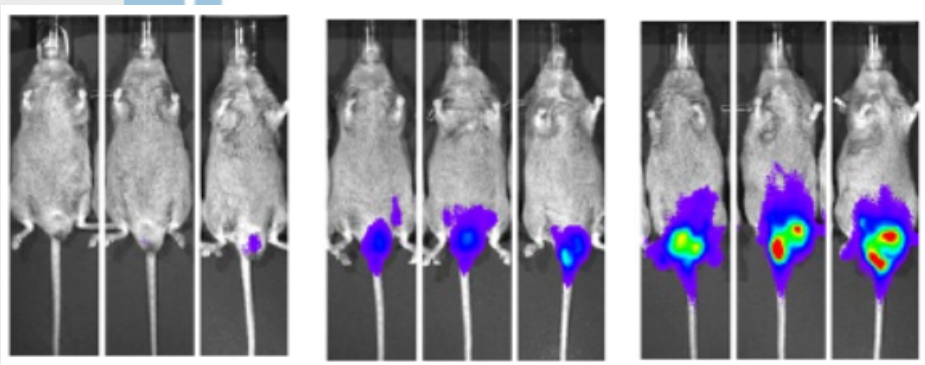
Pseudopregnant female Mice infected

Birth of progeny



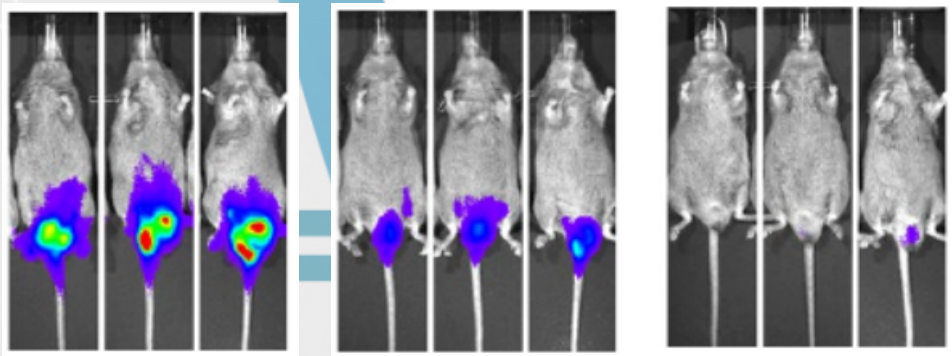
IN VIVO RESULTS

Absence of the FeCl3



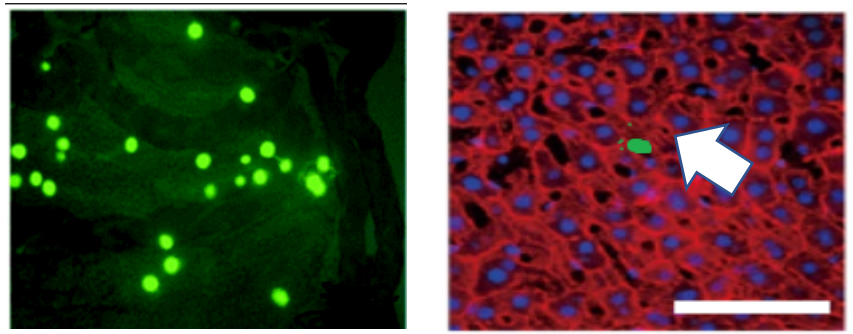
7 days 14 days 21 days

Presence of the FeCl3

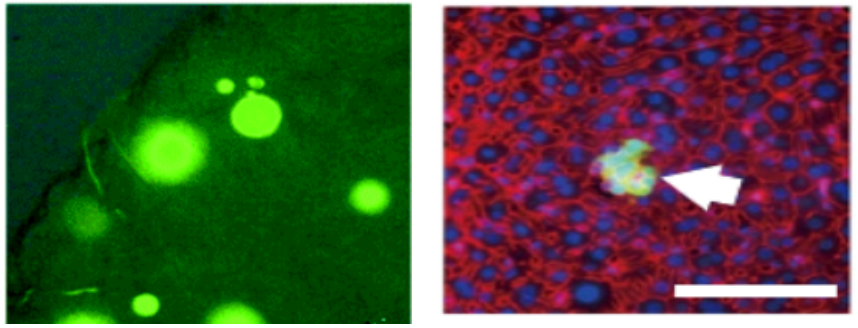


7 days 14 days 21 days

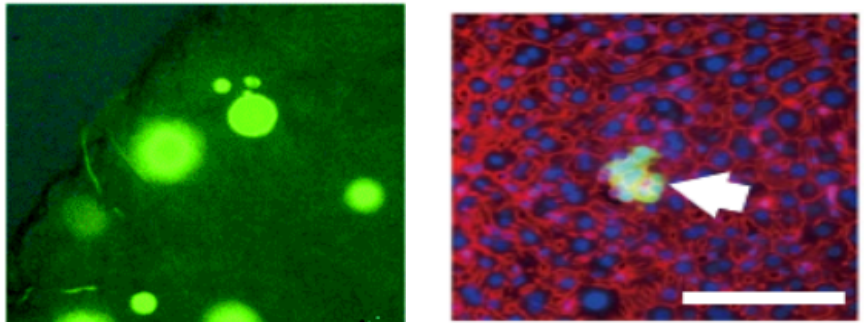
The FeCl3 is involved in the expression of the E.Coli colibactin



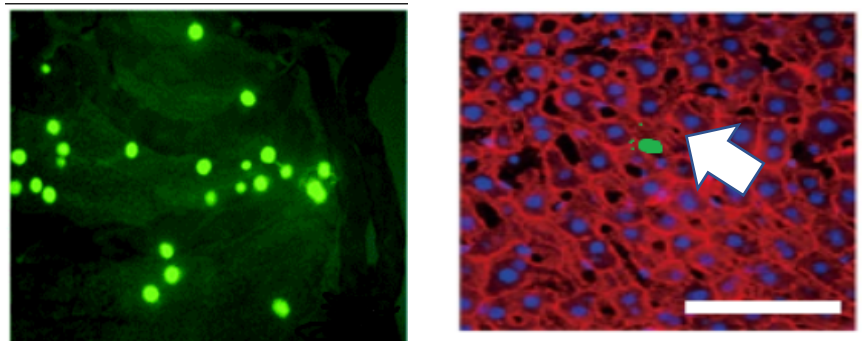
14 days from the infection



21 days from the infection

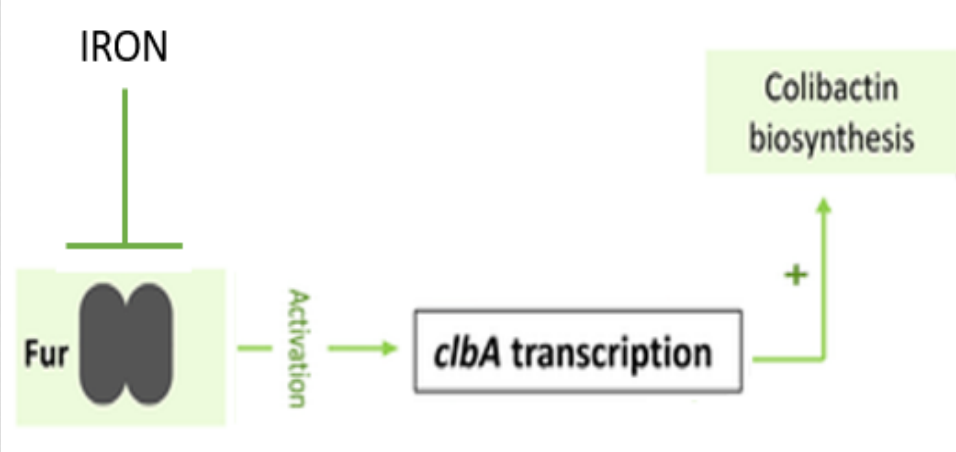


14 days from the infection



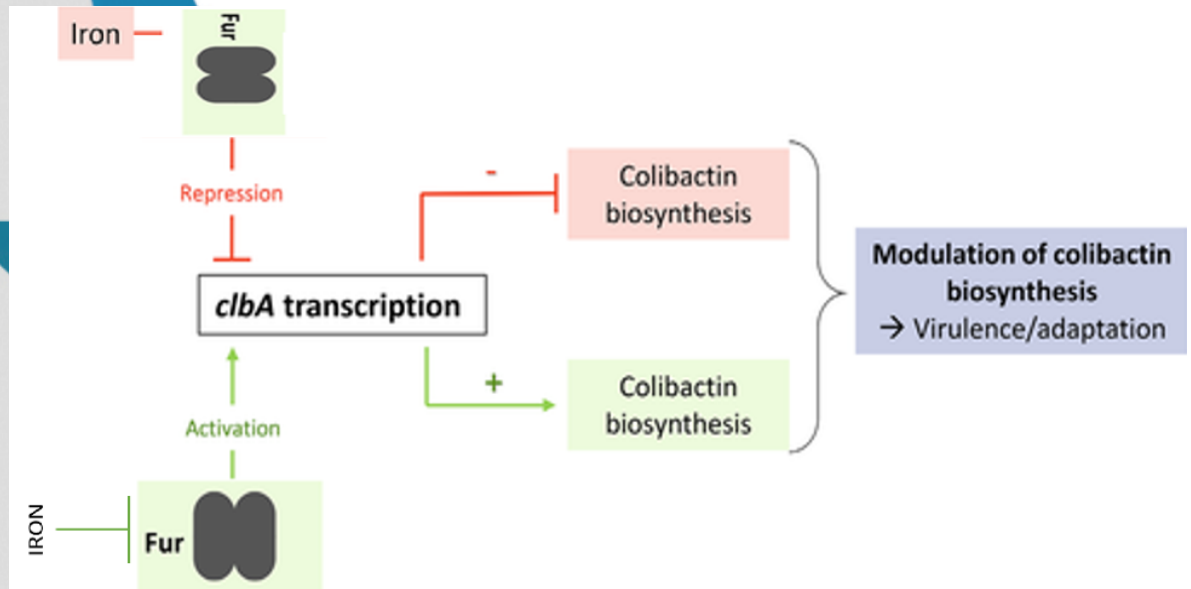
21 days from the infection

CONCLUSIONS



The Fur protein, in absence of iron, active the promoter of clbA gene permitting the colibactin biosynthesis

The Fur protein, in presence of iron, doesn't able to bind the promoter of clbA gene causing the repression of colibactin biosynthesis



The presence/absence of Iron is involved to regulation of clbA protein by using Fur protein. The iron cause the moludation od colibactin biosynthesis by a mechanism based on ***REGULATION BY METABOLITE***.

Materials and costs

- ❑Ferric chloride solution (Sigma Aldrich) 36,90€ per 100g bottle
- ❑Escherichia coli Vitroids™ (Sigma Aldrich) 62,00€ per sample
- ❑Emsa kit (Thermo Scientific™ 20148)518,00€
- ❑Chromatographic column (GE Healthcare) 500€
- ❑Plates (Sigma Aldrich) 100€
- ❑Mice: from 4 € to 20 €
- ❑GFP yeast reporter plasmid (sigma Aldrich) 326€
- ❑Costs of lab manteinance and materials

REFERENCES

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